

# Honey Bee of Serbia

## A Population-Genetic Perspective



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# Apis mellifera - EUROPEAN HONEY BEE

Economically and ecologically the most important

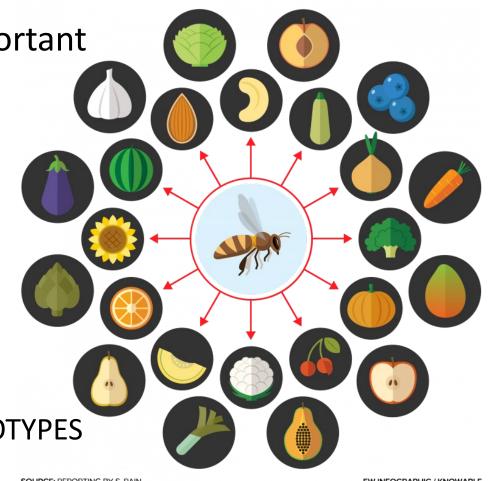
key crop pollinator

Native to Africa, Asia, and Europe

Present day – Cosmopolitan

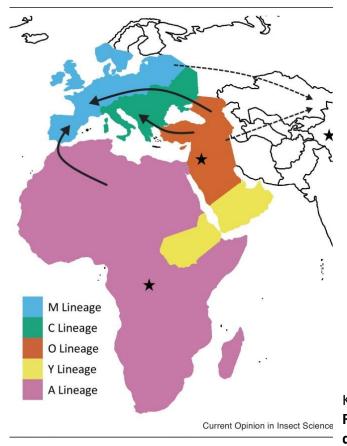
• ≈30 described subspecies

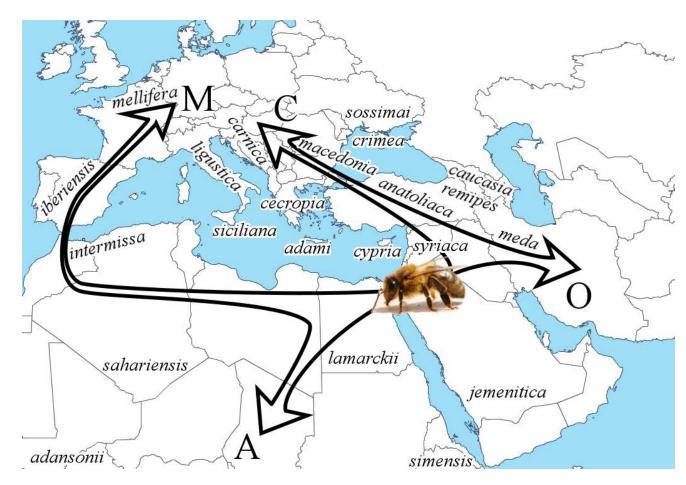
Within the subspecies, there are various ECOTYPES





### Five evolutionary lines





K.A. Dogantzis, A. Zayed (2019)

Recent advances in population and quantitative genomics of honey bees

Curr. Opin. Insect Sci., 31 pp. 93-98

Gil Leclercq, Nicolas Gengler & Frédéric Francis (2018) **How human reshaped diversity in honey bees (***Apis mellifera* **L.): a review**Entomologie Faunistique – Faunistic Entomology 71



Central part of the distribution area of the C Line

Literature data

2 SUBSPECIES in Serbia:

Apis mellifera carnica – North-West Apis mellifera macedonica – South-East

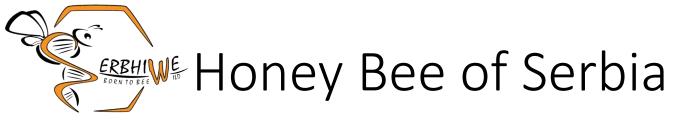
**3 ECOTYPES** characteristic for specific geographic regions:

Banatski, Sjenica-Pešterski and Timočki

mtDNA analysis – more than 7 Haplotypes

Microsatellite analysis – clinal distribution North-South and East-West

Material for last genetic analysis was collected between 2005-2012 2005-2007 Stevanovic, Nedić 2009-2010, Munoz 2007-2012



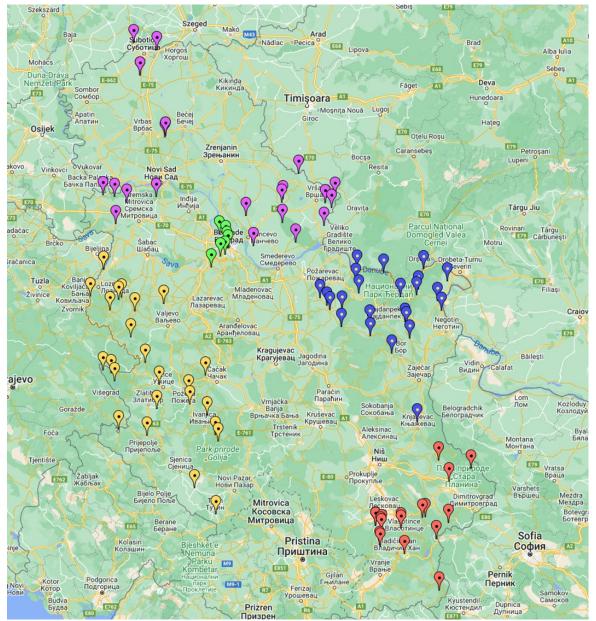
### Managed honey bees – APIARIES

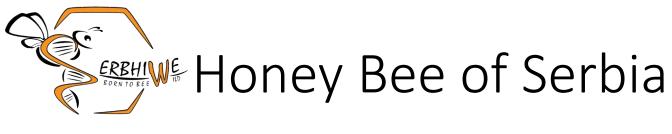
#### Terms:

- The older apiaries
- Stationary apiaries
- Without introduction of Queens for a long period of time

#### **GOALS:**

- Describing the genetic variability of honey bees of our country
- Establish a picture of population structure of Serbian honey bees
- Recognition of locally adapted ecotypes and populations





### Managed honey bees — 102 APIARIES

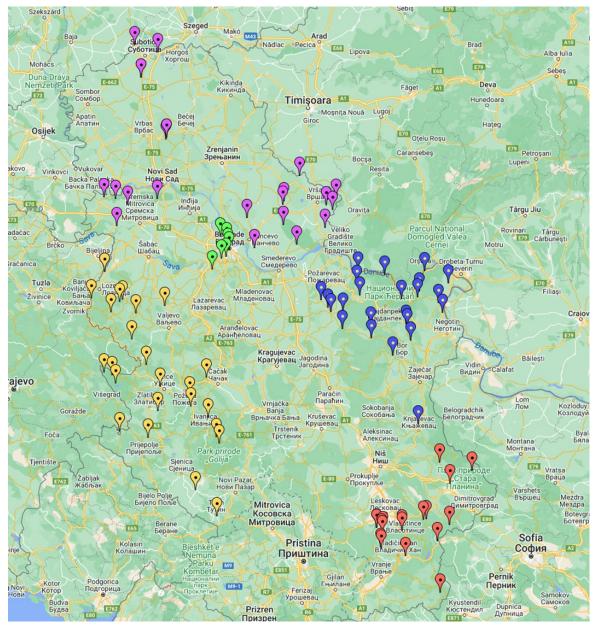
NORTH (Vojvodina): Palić, Fruška Gora, Deliblatska peščara, Vršac

SOUTH: Leskovac, Stara planina, Vlasina, Grdelička gorge

WEST: Ovčarsko-kablarska gorge, Valjevo, Tara, Uvac, Sjeničko-pešterska visoravan

EAST: Đerdapska gorge, Homolj, Kučaj, Negotin

**BELGRADE** 





# SERBHIWE project

Two goals in research

#### **MANAGED HONEY BEES**

- apiaries



#### **FREE-LIVING HONEY BEES**

natural habitats and urban environments



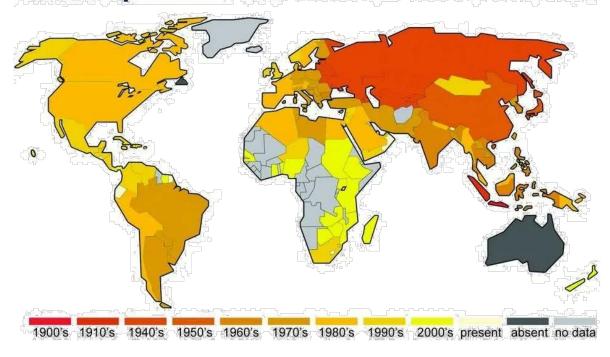


 the disappearance of wild honey bees in the natural habitats of Europe





### Global spread of V. destructor in A. mellifera









- Wild or
- Feral



# Free-living honey bee colonies sampling methods

1. Beelining method - "following the line of bee flight"
Catching, marking, releasing and waiting, moving in the direction of the bees' flight









sampling methods

### 2. Searching for information

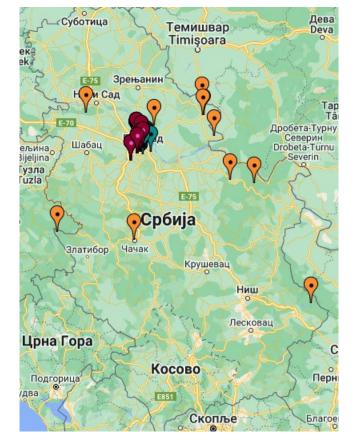


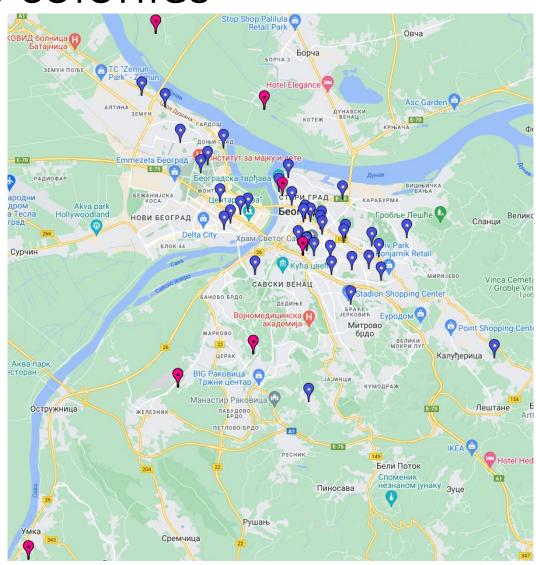






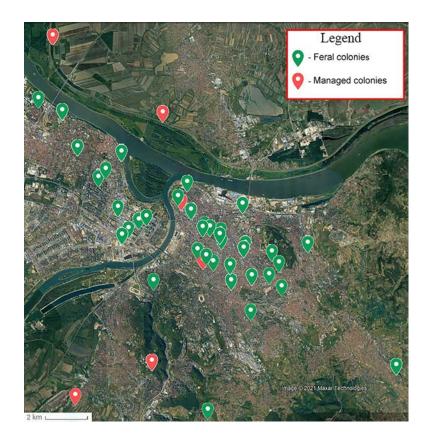
natural habitats and urban environments (56)







database of feral societies on the territory of Belgrade







Over 930 honey bee colonies (HIVES)
542 workers have been selected FOR GENETIC ANALYSES
+ 56 free-living workers

### **2 types of GENETIC MARKERS:**

1. **Mitochondrial DNA (mtDNA)** *tRNA*<sup>leu</sup>-cox2 intergenic region

COI

2. **Microsatellites** - 14 Loci A7, A8, A14, A24, A28, A35, A43, A79, A88, A107, A113, Ap43, **Ap249**, **B124** 





## MtDNA: RFLP analysis on the COI gene segment

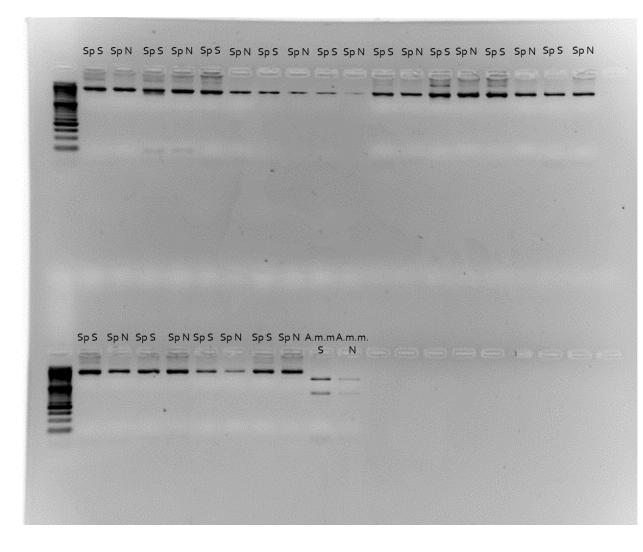
to distinguish two subspecies

A. m. carnica from A. m. macedonica

COI Segment: 1029 bp

2 Restriction Enzymes: *Ncol* and *Styl* 

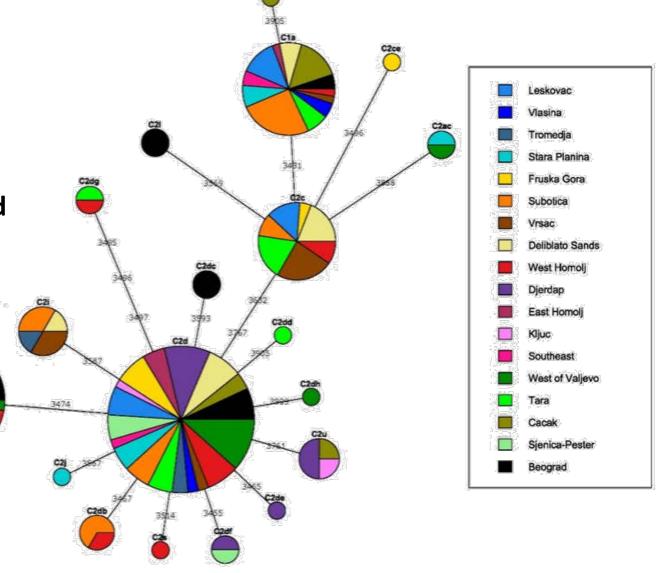
- RFLP analysis showed the presence of *A. m. carnica* subspecies only
- Absence of A. m. macedonica subspecies from its historic range of distribution in southern Serbia





mtDNA: tRNA<sup>leu</sup>-cox2 intergenic region

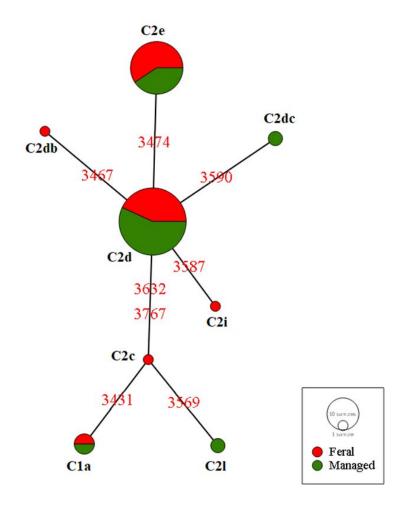
- 20 haplotypes
- 10 the first time described
- In a study Stevanović et al. 2010\* only one mitochondrial haplotype was detected – C2d
- the most frequent **C2d, C2e, C2c, C1a** represented in all regions of Serbia
- high gene flow

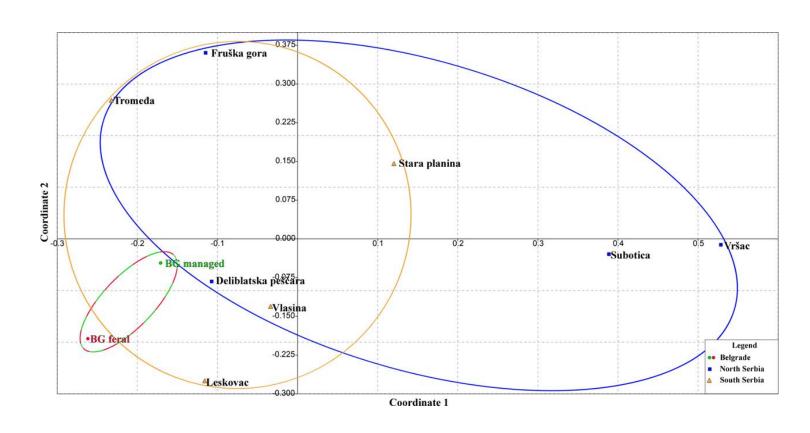


<sup>\*</sup> Stevanovic J, Stanimirovic Z, Radakovic M, Kovacevic SR (2010), Biogeographic study of the honey bee (*Apis mellifera* L.) from Serbia, Bosnia and Herzegovina and Republic of Macedonia based on mitochondrial DNA analyses, Genetika 46(5), 685-91



# mtDNA — BG feral and managed honey bee





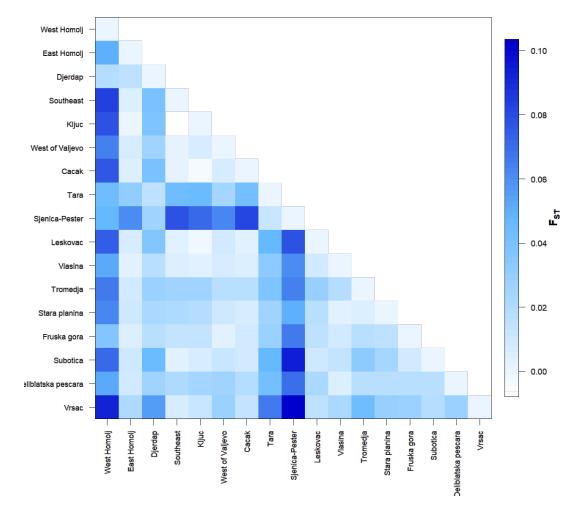


# Microsatellites — managed honey bees

#### Matrix of pairwise F<sub>ST</sub>

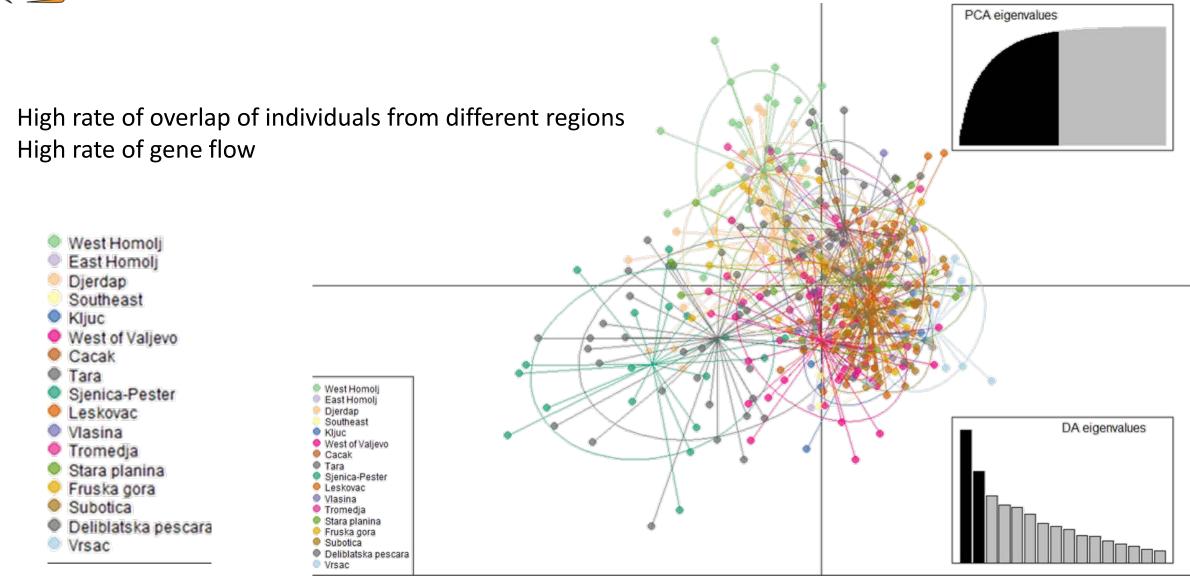
#### PARAMETERS OF GENETIC DIVERSITY

- Uniform
- Expected heterozygosity higher than observed
- The highest average diversity of genes by loci: Sjenica-Pešter region

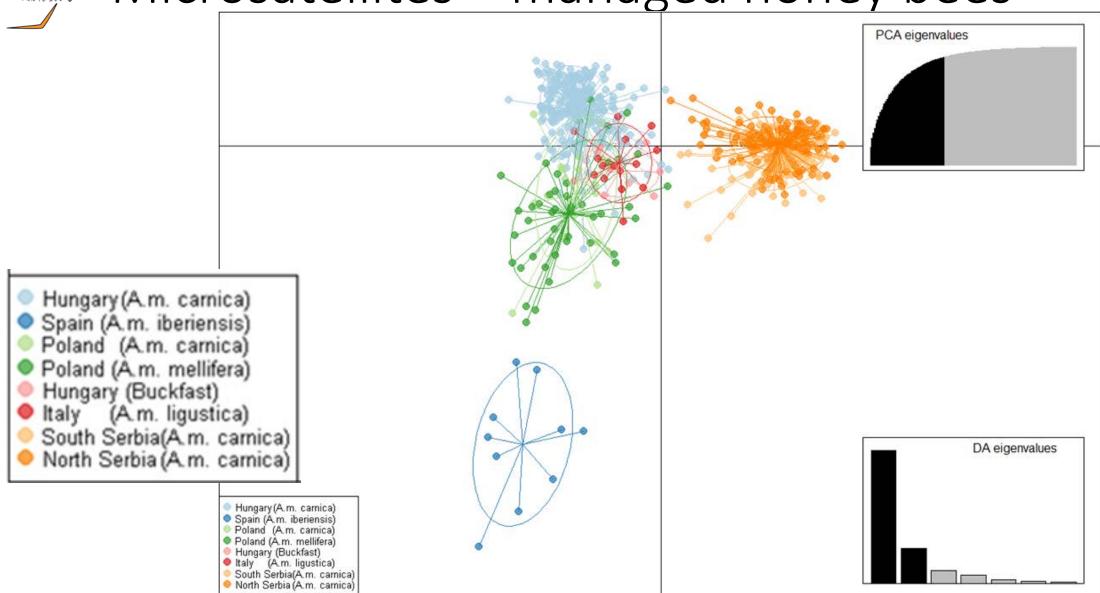




# Microsatellites – managed honey bees

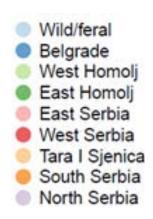


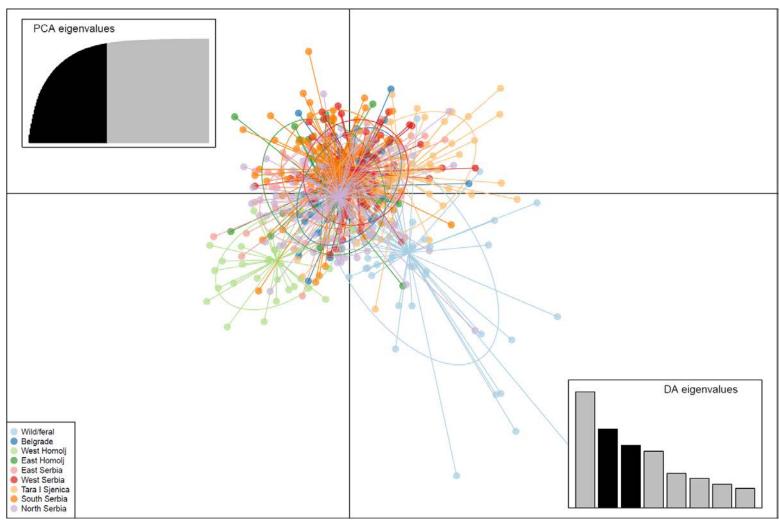
Microsatellites – managed honey bees





Microsatellites - managed vs. feral





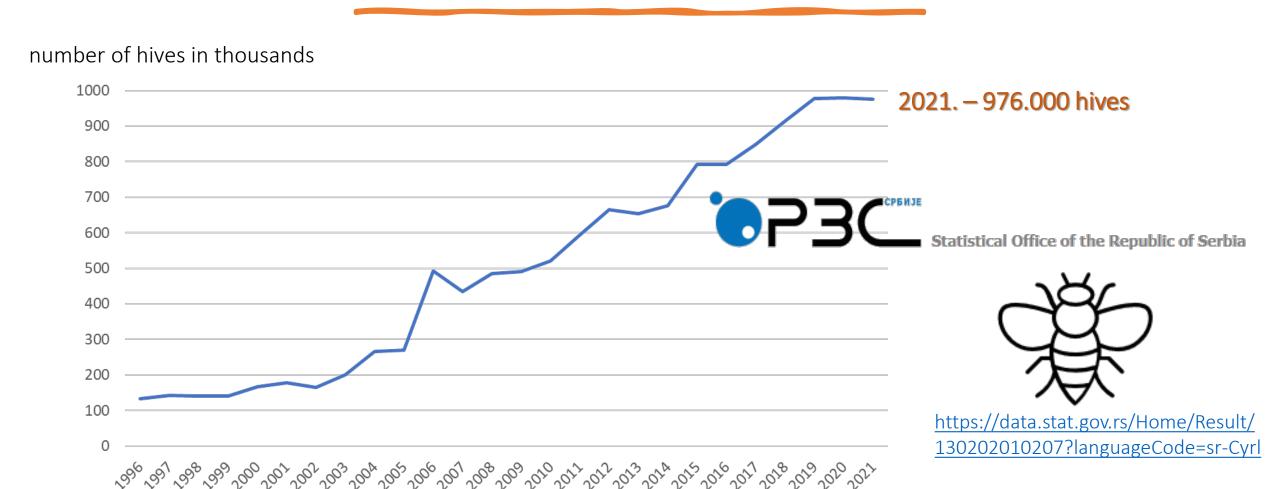


# Microsatellites - Serbian honey bees



Coordinate

### Some possible explanations



### Some possible explanations

Change in beekeeping practices – the traditional beekeeping way has been lost

### **Modern Beekeeping:**

- Queen importation
- Large-scale and long-distance migratory beekeeping
- Commercial honey bee breeding and selection for desired traits







### Some possible explanations

# Law on Animal Husbandry of the Republic of Serbia

(Official Gazette of RS, No. 41/2009, 93/2012 and 14/2016, Article 70)

allows the cultivation of Carniolan honey bee

### Especially preservation of biodiversity bee races Apis mellifera carnica

Article 70.

In order to preserve biodiversity bees Apis mellifera carnica in the territory of Republic of Serbia is not allowed breeding and trade with breeding material from other races of bees.











#### **GREATER GENETIC VARIABILITY**

- \* Reservoir of genetic diversity
- Subject to natural selection
- Better adapt to diseases, pathogens, and adverse, challenging habitat conditions.





# Honey Bee of Serbia – Population-Genetic Perspective RESULTS

- MtDNA lineages characteristic for A.m.macedonica subspecies could not be detected
- MtDNA genetic diversity has substantially changed and in free-living colonies it exhibits a bit higher values of genetic diversity
- Genetic differentiation among different regions is non-existent or weak
- Sjeničko-pešterski ecotype could most likely be differentiated
- Free-living honey bees colonies of Belgrade represent a stable self sustainable population (with small or non detectable input of swarms from managed apiaries)
- Current situation calls for a change in honey bee management









Article

Unprecedented Density and Persistence of Feral Honey Bees in Urban Environments of a Large SE-European City (Belgrade, Serbia) †

Jovana Bila Dubaić 10, Slađan Simonović 2, Milan Plećaš 10, Ljubiša Stanisavljević 1,\*0, Slobodan Davidović 30, Marija Tanasković 30 and Aleksandar Ćetković 10





Proceedings

Microsatellite Analysis of Apis mellifera from Northern and Southern Parts of Serbia †

Marija Tanasković 1\*, Aleksandra Patenković 1, Katarina Erić 1, Pavle Erić 1, Ljubiša Stanisavljević 2 and Slobodan Davidović





MtDNA Analysis Indicates Human-Induced Temporal Changes of Serbian Honey Bees Diversity

Marija Tanasković 1,\*0, Pavle Erić 10, Aleksandra Patenković 10, Katarina Erić 1, Milica Mihajlović 2, Vanja Tanasić <sup>2</sup>, Ljubiša Stanisavljević <sup>3</sup> and Slobodan Davidović <sup>1</sup>





Further Evidence of Population Admixture in the Serbian **Honey Bee Population** 

Marija Tanasković 1,\* (a. Pavle Erić 10), Aleksandra Patenković 10), Katarina Erić 10), Milica Mihajlović 2, Vanja Tanasić <sup>2</sup>, Szilvia Kusza <sup>3</sup>, Andrzej Oleksa <sup>4</sup>, Ljubiša Stanisavljević <sup>5</sup> and Slobodan Davidović <sup>1</sup>



**scientific** reports

### **OPEN** Urban ecosystem drives genetic diversity in feral honey bee

Aleksandra Patenković¹™, Marija Tanasković¹, Pavle Erić¹, Katarina Erić¹, Milica Mihajlović², Ljubiša Stanisavljević3 & Slobodan Davidović1



#### Александра Патенковић НАУЧНИ ПРОЈЕКАТ SERBHIWE







Bee World

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/tbee20

Insight into Free-Living Honey Bee Population in Serbia's Capital - A COLOSSal Walk around Belgrade

Jovana Bila Dubaic´, Raffaele Dall'Olio & Slobodan Davidovic´

STRUČNI RAD

### Genetička raznovrsnost i budućnost medonosne pčele u Srbiji

Autori: Aleksandra Patenković<sup>1</sup>, Pavle Erić<sup>1</sup>, Katarina Erić<sup>1</sup>, Marija Tanasković<sup>1</sup>, Ljubiša Stanisavljević<sup>2</sup> Slobodan Davidović<sup>1</sup>







Слободан Давидовић

ПРОЈЕКАТ SERBHIWE: РЕЗУЛТАТИ И ПОУКЕ





#### **Notable Articles**

Insect Societies and Sociality

Bayesian Multi-Targets Strategy to Track Apis mellifera Movements at Colony Level

Jordão N. Oliveira, Jr. et al.

Insect Societies and Sociality

Geographical Variation of Honey Bee (Apis mellifera L. 1758) Populations in South-Eastern Morocco: A Geometric Morphometric Analysis

Abdessamad Aglagane et al.

Insect Societies and Sociality

Translocation of Tebuconazole between Bee Matrices and Its Potential Threat on Honey Bee (Apis mellifera Linnaeus) Queens

Risto Raimets et al.

Insect Physiology, Reproduction and Development

Functional Properties and Antimicrobial Activity from Lactic Acid Bacteria as Resources to Improve the Health and Welfare of Honey Bees

Massimo lorizzo et al.

Advanced in Honey Bee and Apitherapy

Low-Level Fluvalinate Treatment in the Larval Stage Induces Impaired Olfactory Associative Behavior of Honey Bee Workers in the Field

Chong-Yu Ko et al.

Insect Societies and Sociality

Sex-Specific Regulatory Systems for Dopamine Production in the Honey Bee Ken Sasaki et al.

Insect Societies and Sociality

Differential Expression of Major Royal Jelly Proteins in the Hypopharyngeal Glands of the Honeybee *Apis mellifera* upon Bacterial Ingestion

Yun-Hui Kim et al.

Insect Societies and Sociality

<sup>1</sup>H NMR Profiling of Honey Bee Bodies Revealed Metabolic Differences between Summer and Winter Bees

Saetbyeol Lee et al.

Insect Societies and Sociality

Effects of Thiamethoxam-Dressed Oilseed Rape Seeds and Nosema ceranae on Colonies of *Apis mellifera iberiensis*, L. under Field Conditions of Central Spain. Is Hormesis Playing a Role?

Elena Alonso-Prados et al.

Selected Papers from the 1st International Electronic Conference on Entomology

Further Evidence of Population Admixture in the Serbian Honey Bee Population

Marija Tanasković et al.



International **Golden Bee Award** Slovenia, 2023.





### SERBHIWE project

July 2020. – September 2022.

#### **TEAM** members:

- dr Slobodan Davidović (PI)
- dr Marija Tanasković
- dr Aleksandra Patenković
- Katarina Erić
- Pavle Erić

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prof. dr Ljubiša Stanisavljević

<u>Department of Invertebrate Zoology and Entomology</u> Faculty of Biology, University of Belgrade



